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DDT is still a problem in developed countries: the heavy pollution of Lake Maggiore

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Abstract

The Zebra mussel (*Dreissena polymorpha*), one of the most widely used bioindicators of persistent organic pollutants, trace metals and radionuclides in several worldwide freshwater ecosystems, has been used to monitor DDT contamination trends in Lake Maggiore since 1996, caused by industrial effluents on a tributary of the River Toce, one of the major affluents of the lake. *Dreissena* specimens were collected at two sampling sites, one within the Baveno Bay, where the River Toce flows, and the other outside (Villa Taranto). Total DDT levels (3119.6 ng/g lipids at Baveno and 1351.2 ng/g lipids at Villa Taranto) in the soft tissues of the Zebra mussel decreased at both stations by about 30–50% in the first year after the closure of the chemical plant reaching an almost steady-state condition. The high concentrations measured in Zebra mussel specimens of Baveno Bay in 2000 (1947 ng/g lipids) and the percentage of *pp*′DDE in comparison with total DDT concentration, which showed a slight increase in the last years, clearly indicate that a contamination source is still present, deriving probably from the lacustrine sediments and the River Toce. Data show that the environmental risk is very high within the Baveno Bay and the recovery time should be longer than in the other parts of the lake, where DDT levels in *Dreissena* are presently two times higher than those measured in the other Italian subalpine lakes.

Keywords: Zebra mussel; DDT; Biomonitoring; Freshwater ecosystems; Lake Maggiore

1. Introduction

Persistent organic pollutants (POPs) are highly stable organic compounds used as pesticides in agriculture and in industry, or generated unintentionally as the by-product of combustion and industrial processes. POPs have chemical and physical properties which cause numerous problems to the environment, wildlife and human health: POPs are lipophilic compounds that can bioaccumulate in lipid tissues and, owing to their persistent and semi-volatile nature, can travel long distances and condense over the colder and more remote areas of the world (Kurtz, 1990; Cotham and Bidleman, 1991). As a result of these properties, there is growing concern over the toxic effects of these compounds on biota, in particular on the higher species of the food chain, even at extremely low environmental levels. Some of these chemicals are endocrine disruptors and various dangerous effects such as eggshell thinning, behavioural changes, impaired male reproductive ability and estrogenic activity interfering with sex hormones have been confirmed (Kelce et al., 1995). The problem of endocrine disruptors is one of the five priority research areas established by the Committee on the Environment and Natural Resources of the United States (Kavlock, 1999) and some detailed research strategies have also been put forward by the European Union.

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Until the 1970s, DDT was one of the most widely used pesticides in many Western countries, although a number of studies showed several adverse environment effects and a proven hazard to non-target organisms such as fish and birds. Several legislative decrees were ratified in Europe to regulate its use, but no restrictions have so far been set for the production of this insecticide. In Italy, agricultural use of DDT was banned in 1978, excepted for particular applications in floriculture and zootechnics. Recently, a treaty to control, reduce or eliminate discharges, emissions and losses of several POPs was signed by the European Community. The protocol regulates 12 chemicals, of which most are subject to an immediate ban. These include aldrin, chlordane, DDT, dieldrin, endrin, eptachlor, mirex, toxaphene, polychlorinated biphenils (PCBs), hexachlorobenzene, dioxins and furans. Other protocol obligations include the application of best available technology to limit air emissions from major stationary sources of dioxins (PCDDs), furans (PCDFs), HCB and polycyclic aromatic hydrocarbons (PAHs). However, a health-related exemption has been granted for DDT, which is still needed in many countries to control malarial mosquitoes. Similarly, in the case of PCBs, which have been widely used in electrical transformers, governments may maintain existing equipment in a way that prevents leaks until 2025 to give them sufficient time to arrange for PCB-free replacements.

Notwithstanding the legal restrictions governing the use of several POPs in Western countries, some have recently been found in various American and European aquatic ecosystems (Mersch et al., 1992; Bruner et al., 1994; Roper et al., 1996; Hendriks et al., 1998; Gundacker, 1999; Binelli et al., 2001a,b). Environmental problems of POPs are still persistent, as shown by the detection of DDT contamination in Lake Maggiore in 1996, the second-largest Italian lake by area and volume—an outstanding example of how a pollution problem associated with a chemical whose use, but not production, was banned two decades ago can arise. A previous study conducted in Lake Maggiore (Ceschi et al., 1996) showed very high levels of DDT in several species of fish, beyond the legal limit for edible fish. The commercial fishing of some species in this lake was halted in June 1996. The source of the pollution was traced back to a chemical plant (CIPAIS, 1999), which both produced and discharged DDT over several years into the River Marmazza, a tributary of the River Toce, in turn one of the major affluents of Lake Maggiore (Fig. 1).

We carried out a 5-year biomonitoring operation (1996–2000), using the bivalve *Dreissena polymorpha* to measure DDT changes in Baveno Bay, where the River Toce flows, and the spread of contamination outside the bay. Wide distribution, continuous availability throughout the year, adequate body size, firm site attachment capability of the bissus, ease of sampling, good salinity tolerance and relatively high longevity make this mollusc particularly appropriate for use as a bioindicator. The Zebra mussel has already been used to assess the contamination of freshwater ecosystems by trace metals (Johns and Timmerman, 1998; Gundacker, 1999), organotin compounds (Becker et al., 1992) and POPs (Mersch et al., 1992; Bruner et al., 1994; Hendriks et al., 1998).

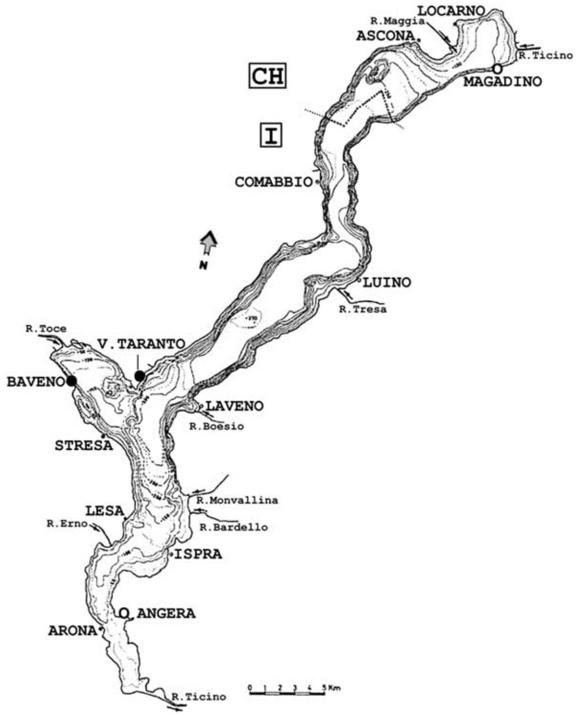

Fig. 1. Lake Maggiore basin with 5-year sampling sites (dark circles) and the other two stations of 1998 operation (white circles).

2. Sampling and study sites

Lake Maggiore is one of the largest European lakes (volume $37\,500 \times 10^6$ m^3 and area 212.5 km^2). Sampling was carried out between 1996 and 2000 at two stations: Baveno, close to the mouth of River Toce, within Baveno Bay, and Villa Taranto, located outside the bay. Two other sites were sampled during a previous operation only, carried out on *Dreissena* adult specimens, with a shell length greater than 15 mm that were collected by a scuba-diver at Baveno and Villa Taranto in June. Specimens were transported to the laboratory in refrigerated bags and frozen at −18 °C before chemical analysis.

3. Methods

A pool of 30–40 animals were defrosted, the shell and bissus removed, the soft tissues freeze-dried for about 24 h and then homogenized by a tissue grinder. A soft tissue sample (1.5 g) was then extracted with *n*-hexane/acetone (1:1 vol.) in a Soxhlet for 8 h. The organic phase was evaporated to dryness, weighed to determine lipid content and digested with H_2SO_4 (98%) for 24 h. DDTs were recovered from the digested lipid fraction by small aliquots of n-hexane and run on a 35×1 cm Florisil column (Lazar et al., 1992): *op*′DDE, *pp*′DDE, *op*′DDT, *pp*′DDT, HCB and PCBs including mono-ortho substituted were eluted with 70 ml of n-hexane (fraction 1); *op*′DDD, *pp*′DDD, lindane and non-ortho substituted PCBs were eluted with 60 ml of a mixture of dichloromethane–hexane (15:85 vol.) (fraction 2). The eluates were concentrated to 1 ml. Finally, samples (1 μl) were injected into a Carlo Erba 8000 GC equipped with electron capture detector (ECD), a column Varian CP-sil 19 (length = 50 m, i.d. = 0.25 mm, film thickness = 0.20 μm) and Chrom-Card software. Analyses were conducted in duplicate. DDTs were finally quantified by comparison of peak areas of individual compounds with a reference standard. A ring-test among several laboratories carried out in 1996 showed that the percentage recovery of this procedure was up to 80% for each DDT relative and the detection limit was 1 ng/g of dry weight.

We used the Cluster analysis (STATISTICA® software) to measure any similarity among the various years. The single DDT relatives concentration and *pp*′DDT/*pp*′DDE ratio for each year were used as cases and the different years as variables.

4. Results

The Zebra mussel reproductive cycle trend in the Southern Alps seems to have an over-winter development phase of gametes, a spawning stage during the spring-summer season and finally an inactive gonad period (Bacchetta et al., 2001). Even if all the Zebra mussel specimens were collected in the same period at the end of spring to reduce the differences of DDT concentration through physiological or environmental changes already detected elsewhere (Binelli et al., 2001a), differences among lipid percentages were noticed over the 5-year period (Table 1). On the other hand, the use of an aquatic bioindicator implies an intrinsic variability of its physiological and ecological behaviour, due to different hydrological conditions and seasonal weather changes. Thus, the results were expressed as lipid-based concentrations in order to eliminate differences due to nutritional condition or reproductive stage (Binelli et al., 2001a).

Table 1
Sample characteristics of mussel specimens and date of sampling

Date of sampling	Baveno		Villa Taranto	
	Percentage of lipids	Average length (cm)	Percentage of lipids	Average length (cm)
17 June 1996	11.5	2.0	12.2	2.0
18 June 1997	7.0	2.3	12.6	1.8
18 June 1998	9.8	2.0	10.0	2.0
16 June 1999	11.4	2.1	12.4	2.2
19 June 2000	16.3	1.9	18.9	1.9

Lipids found in Zebra mussels collected in 1997 at Villa Taranto were twice as high as those of Baveno because an earlier gamete release and histological alterations were observed in the female reproductive apparatus compared to those collected from the less contaminated area (Binelli et al., 2001b).

DDT pollution was higher before the closure of the chemical plant both within and outside Baveno Bay (Tables 2 and 3), but the sum of relatives in Zebra mussel specimens of Baveno in 1996 (3119.6 ng/g lipids) was more than double those collected at Villa Taranto (1351.2 ng/g lipids).

Five-year trends revealed quite similar variations between the two sampling sites: further to the interruption of DDT releases, high decreases of about 32% and 55% were measured in 1997 inside and outside the bay. This trend was already interrupted 1 year later at Villa Taranto, increasing by approximately 34%, while a decrease of 29% was obtained at Baveno. The sum of relatives revealed a slight increase in 1999 in the two stations, mainly due to a rise in *pp*′DDE concentrations. Finally, the sum of DDTs in 2000 was more or less the same as those registered in 1997 at the two sampling sites.

Table 2
Levels (ng/g lipids) of DDT residues in soft tissues of Zebra mussel sampled at Baveno

	1996	1997	1998	1999	2000
op′DDE	196.9	47.5	66.9	25.8	177.4
pp′DDE	901.2	635.6	484.2	761.6	655.2
op′DDD	325.1	314.0	231.6	170.6	245.0
pp′DDD	561.6	520.7	225.9	290.7	337.2
op′DDT	451.5	137.8	205.8	125.2	206.3
pp′DDT	683.3	452.6	292.8	313.7	325.7
Sum of relatives	3119.6	2108.2	1507.2	1687.5	1946.7
pp′DDT/*pp*′DDE	0.76	0.71	0.60	0.41	0.50

Table 3
Levels (ng/g lipids) of DDT residues in soft tissues of Zebra mussel sampled at Villa Taranto

	1996	1997	1998	1999	2000
op′DDE	42.5	2.9	53.3	9.1	22.7
pp′DDE	319.8	156.3	305.3	450.3	242.8
op′DDD	190.3	159.5	126.4	100.8	112.6
pp′DDD	400.9	179.8	196.1	231.5	231.2
op′DDT	79.9	36.8	31.4	25.4	29.2
pp′DDT	317.8	77.3	110.0	64.8	84.3
Sum of relatives	1351.2	612.6	822.5	882.0	722.8
pp′DDT/*pp*′DDE	0.99	0.49	0.36	0.14	0.35

Also the *pp*′DDT/*pp*′DDE ratio (Tables 2 and 3) revealed a decreasing trend until 1999, but a clear rise in 2000 (0.50 at Baveno and 0.35 outside the bay).

5. Discussion

There is a scarcity of published data pertaining to DDT studies over the past 10 years, either regarding monitoring or its toxic effects on wildlife or humans, while the recent discovery of its estrogenic properties has generated a renewed interest. This interruption is probably due to the legal restrictions on the use of this organochlorine insecticide in developed nations and the falling contamination trend observed in Western aquatic ecosystems. On the contrary, several studies on DDT biomonitoring have been carried out in the last decade in numerous developing countries, where some POPs are still used (Tanabe et al., 2000). Even though DDT biomonitoring of coastal zones using marine molluscs has been commonly used, little data is available on freshwater bioindicators, in particular for the Zebra mussel.

When compared with different surveys in other bivalve species from various worldwide locations (Table 4), total DDT levels found in Lake Maggiore are higher than those measured in developed nations, except for similar values measured in the Nordic seas, another heavy polluted aquatic environment, but are of the same order as those measured in developing countries.

The heavy DDT contamination in Lake Maggiore is particularly significant as it is an example of how a chemical that was banned in a specific developed country many years beforehand can be harmful to a large aquatic environment and subsequently to human health. Histological studies carried out in 1997 (Binelli et al., 2001b) showed that a significant percentage of specimens sampled inside the Baveno Bay had a marked oocyte degeneration and released gametes earlier than those sampled at a nearby reference station outside the bay, suggesting a possible endocrine-disrupting effect of DDTs.

A 5-year study appears to be sufficient to investigate the evolution of the contamination, in order to formulate different hypotheses on the expectations, pathways, transformations and eventual other sources of this insecticide.

The half-life of *pp*′DDT in fish is about of 8 months and that of *pp*′DDD and DDE is much longer (about 7 years), while the measured half-life of *pp*′DDT in Zebra mussel soft tissues is 102 and 87 h in unpolluted water (Fischer et al., 1993). Thus, the high DDT levels found in Zebra mussel specimens in June 2000, 5 years after the closure of the chemical plant, reveal a possible input of contaminants from different sources, corresponding to sediment and rivers. On the other hand, a

Table 4
Levels (ng/g) of DDT residues in bivalves collected worldwide

Study area	Species	Habitat	$\sum$DDT (ng/g)	References
Coastal water of USA	*Mytilus edulis*	Marine	5 w.w.	O'Connor (1996)
Perth (Australia)	*Mytilus edulis*	Marine	<1–2 w.w.	Burt and Ebell (1995)
Nordic seas	*Mytilus edulis*	Marine	29–2980 lipids	Gustavson and Jonsson (1999)
Arcachon Bay (France)	*Mytilus edulis*	Marine	23.4 d.w.	Thompson et al. (1999)
All Saints Bay (Brazil)	*Brachidontes exustus*	Marine	2.1–44.0 w.w.	Tavares et al. (1999)
India	*Perna viridis*	Marine	0.9–40 w.w.	Tanabe et al. (2000)
Philippines	*Perna viridis*	Marine	0.19–9.5 w.w.	Tanabe et al. (2000)
Thailand	*Perna viridis*	Marine	1.3–38 w.w.	Tanabe et al. (2000)
Lake Ijsselmer (Holland)	*Dreissena polymorpha*	Freshwater	172 lipids	Reederes and Bij de Vaate (1992)
Lake Erie (USA)	*Dreissena polymorpha*	Freshwater	180 d.w.	Kreis et al. (1991)
Baveno (L. Maggiore, Italy)	*Dreissena polymorpha*	Freshwater	13.31–34.76 w.w.	Present study
Villa Taranto (L. Maggiore, Italy)	*Dreissena polymorpha*	Freshwater	7.72–16.46 w.w.	Present study

d.w.: dry weight basis and w.w.: wet weight basis.

Table 5
Annual input and basin contribution in 1998 for the main tributaries of Lake Maggiore (CIPAIS, 1999)

Tributary	Area (km^2)	Input (kg/y)	Basin share (g/km^2 y)
Toce	1546.8	2.10	1.36
Verzasca	236.8	0.21	0.89
Maggia	509.4	0.67	1.31
Ticino	1616.2	1.39	0.86
Vevera	21.4	0.01	0.64
Tresa	754.2	0.37	0.49
Margorabbia	94.6	0.05	0.49
Boesio	45.4	0.04	0.79
Bardello	134.3	0.07	0.49

study carried out in 1998 on several tributaries (CIPAIS, 1999) of the lake showed a continued input of DDTs mainly from the River Toce, which discharges more than 2 kg/y of DDT with a basin contribution of 1.36 g/km^2 y (Table 5).

However, the only point-source identified was the chemical plant located on the River Toce, and the input of these tributaries suggests that Lake Maggiore behaves as reservoir of the insecticide and its metabolites. DDTs, like the other semi-volatile compounds, can evaporate from the water column, be transported by atmospheric currents, and fall again with the rain or snow into the surrounding watershed, covering an area of more than 6,500 km^2. The DDT condensation is likely to occur also because of the altitude of several mountains, which are among the highest in the Alps.

This DDT circulation can be confirmed by the high concentration of the total DDT measured in the bay 5 years after the closure of the chemical plant, as also shown by other analysis conducted for sediments and fish (CIPAIS, 1999). Research into DDT contamination in wet and dry depositions and the transport of this insecticide to the surrounding mountains is currently underway.

Figs. 2 and 3 show levels of *pp′*DDTs at Baveno and Villa Taranto to emphasize trends of the most important relatives. We observed a clear decrease in DDTs inside the bay until 1998 (Fig. 2) with a contamination loss passing from 46% for *pp′*DDE to about 57% for the parental compound. This declining trend has been interrupted: in 1999 the concentration of DDD and DDT were similar to the previous year and the final metabolite shows a concentration higher than that measured in 1997. This stop is probably due to a new input of DDT detected in December of 1998 by the Italian Environmental Protection Agency that revealed high levels of this insecticide in the River Toce, caused by an improper recovery operation within the chemical plant itself.

There was also an effective DDT decrease for the Zebra mussel specimens sampled at Villa Taranto, but

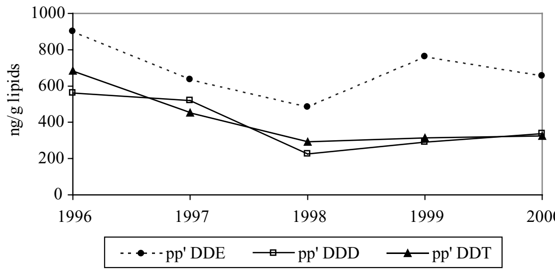

Fig. 2. Temporal trend of *pp′*DDTs at Baveno sampling station.

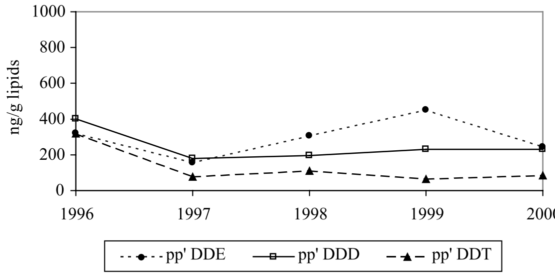

Fig. 3. Temporal trend of *pp′*DDTs at Villa Taranto sampling station.

only for the first year after the closure of the chemical plant (Fig. 3). Thereafter, *pp′*DDT and DDD concentrations remained constant until 2000, while *pp′*DDE in 1999 reached values higher than those measured in 1996, before the end of the insecticide production, confirming that a new contamination problem occurred at the end of 1998.

The differences revealed at Villa Taranto between concentration trends of *pp′*DDT and *pp′*DDE are also due to a possible homogenisation of contamination outside the bay and the consequent transformation of the parent compound into the final metabolite in the water column, facilitated by the lack of continued input of *pp′*DDT from the River Toce, as occurs inside the bay. A biotransformation of DDT by Zebra mussels into DDE might occur, but unfortunately this is not revealed by our data and the percentage of this process for the two lower trophic levels of the food chain is not known. On the other hand, the more homogeneous contamination observed outside the bay is confirmed by DDT concentrations measured by us in 1998 in two other sampling sites, that showed values similar to those of Villa Taranto (Table 3): total DDT concentration was 793 ng/g lipids at Angera, the Southernmost sampling station located near the main outlet of the lake, while the Northernmost station of Magadino, that lies to the north in Switzerland, presented a DDT concentration of 748 ng/g lipids.

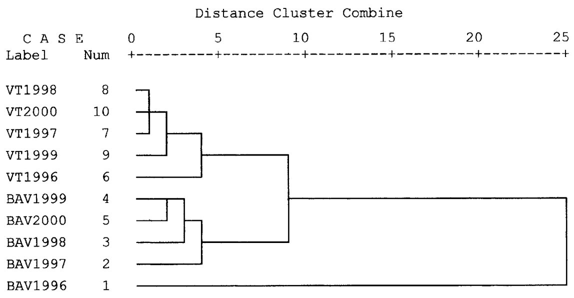

Fig. 4. Hierarchic cluster analysis for DDT concentrations.

Table 6
DDT concentrations (ng/g lipids) in the other great Italian subalpine lakes (1996 operation)

	Como	Iseo	Garda	Lugano
*op'*DDE	7.0	4.3	4.9	18.4
*pp'*DDE	63.6	51.8	45.0	60.1
*op'*DDD	35.5	13.8	13.4	86.7
*pp'*DDD	13.9	11.1	8.3	15.4
*op'*DDT	32.4	14.0	12.8	17.1
*pp'*DDT	41.5	36.0	23.9	35.7
$\sum$DDT	202.4	122.0	238.2	233.4

Fig. 4 shows the hierarchic cluster analysis: two separated groups were identified, depending on the different sampling site, while the DDT contamination measured in 1996 inside the bay is very different from that of the other years, as shown by the higher Euclidean distance calculated for Baveno. Thus, the statistical approach allowed us to confirm the relatively sharp decrease of DDT contamination in Lake Maggiore 2 years after the termination of insecticide production and a steady state situation or even an increase of DDT levels thereafter.

The bay should still be considered an environmental risk area, also because of the release of DDT compounds from the heavily polluted sediments (CIPAIS, 1999). The other parts of the lake may experience faster recovery, even if the actual concentration of DDT relatives at Villa Taranto is still significantly higher ($p < 0.001$) than the background concentration measured in *Dreissena* soft tissues from the other great Italian subalpine lakes during a previous monitoring operation (Table 6).

6. Conclusion

The zebra mussel represents a usable sentinel-organism, which permitted the evaluation of the behaviour of DDT contamination both inside and outside Baveno Bay, showing that recovery time in this area compared to the other parts of Lake Maggiore is almost certainly much longer. DDT is still stored in the chemical plant, where the pollution originated, and in most of the sediments of the drain canal. This leads to possible further inputs into the lake on account of leakages from the plant and floods of the River Toce, which have regrettably been somewhat frequent over the last few years. The chemical plant area is currently undergoing remediation in line with the recently introduced Italian law, but other interventions to limit pollution, including as the drawing of sediments in the bay, are unfeasible due to the average depth of the Baveno Bay (about 100 m) and the huge amount of polluted sediments.

At present, DDT pollution in Lake Maggiore is still a dangerous contamination both for the trophic web, mostly for the higher predators, and for different activities like tourism and fishing and for human health. Bearing in mind that DDT and DDE are assumed to be endocrine disrupters and powerful cancer promoters (Wolf and Toniolo, 1995), it is quite understandable that the medical authorities are investigating the possible risk to the neighbouring population. The pollution of Lake Maggiore is a warning sign for all those developed countries that have not banned the production of organic pesticides and confirms that prevention is the only remedial measure for these kinds of compounds.

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